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Management of post-harvest pathogens of Nagpur mandarin through chemicals, oils, wax and bio-agent

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Abstract
Effect of fungicides, chemical, oils, wax and bio-agent against postharvest pathogens of Nagpur mandarin was evaluated for their inhibitory effect *in vitro*. After 24 hours, Carbendazim @ 0.1 percent was found superior in inhibiting the conidial germination (87.26%) of *Colletotrichum gloeosporioides*, after 48 hours it was (81.11%) and after 72 hours inhibition was (75.90%) recorded. Highest conidial inhibition of *Geotrichum candidum* found in Hydrogen peroxide @ 10 percent was (91.04%), (84.76%) and (77.59%) after 24, 48 and 72 hours respectively. After 24 hours Carbendazim @ 0.1 percent was effective in restriction (88.68%) of conidial germination of *Aspergillus niger* at 48 hours inhibition was (84.78%) recorded and after 72 hours, it was (80.26%). Carbendazim @ 0.1 percent was found superior in inhibiting the conidial germination (92.88%) at 24 hrs of *Penicillium digitatum*, while at 48 hours it was (86.44%) and after 72 hours it was (80.83%). Highest conidial inhibition (85.14%) of *Trichoderma viride* was achieved in Carbendazim 0.1 percent at 24 hrs and after 48 hours it was (78.32%) while, after 72 hours, conidial inhibition was (72.53%).

Keywords: Mandarin, post-harvest, pathogens, conidial germination

Introduction
Citrus (*Citrus reticulata*) is one of the most widely produced fruit globally and grown commercially in more than 137 countries around the world (Ismail and Zhang 2004) [7]. The contribution of the citrus industry to the world economy is enormous, and it provides jobs to millions of people around the world in harvesting, handling, transportation, storage and marketing operations. The importance of citrus fruit is attributed to its diversified use, which is widely consumed either as fresh fruit or as juice. Due to their higher water content and nutrient composition, citrus fruit is very susceptible to infection by microbial pathogens during the period between harvest and consumption (Tripathi and Dubey, 2003). [18] In India, area under citrus crop was 1024 thousand ha and production was 11581 thousand tons and in Maharashtra state area under this crop was 135 thousand ha and production was 742.50 thousand tons (Anonymous, 2015). [1] Citrus fruits are usually quite acidic, in the pH range of 2–4. For this reason, so the most of the decay in harvested fruits is caused by fungi. Contamination and infection by pathogenic fungi occur at different stages in the field and after harvest and usually follows mechanical injury during transportation of the fruits, which allows entry of these micro-organisms. Postharvest decays of fruit can also originate from latent infections occurring in the orchard. Citrus fruits are susceptible to a number of postharvest diseases that cause significant losses during the postharvest phase. Nagpur mandarin is infected by several fungi *viz*., *Penicillium italicum*, *P. digitatum*, *Geotrichum candidum*, *Alternaria alternata*, *A. citri*, *Botryodiplodia theobromae*, *Fusarium* sp., *Glomerella cingulata*, *Aspergillus niger*, *Rhizopus* sp. etc. Post-harvest losses during handling, transport, storage and distribution are the major problems in agrarian economy, especially in perishable fruits. Postharvest diseases cause significant economic losses for the citrus industry during storage, transport and marketing (Naqvi, 2004 [11], Reddy *et al*., 2008 [15], Ladaniya 2008). [9] and Solaimani *et al*., 2009 [16]). The post-harvest handling losses of citrus fruits are 5-10% in most developed countries and 25-30% in developing countries (Maini and Ladaniya, 1999).[10] The infection of these pathogens leads to quantitative and qualitative losses in Nagpur mandarin.
Plant disease control was achieved mainly through the use of fungicides; postharvest decay may increase up to 50% without fungicidal treatments.

Although decay can be reduced to 5-10% with postharvest fungicides. Isoprothiolane, Trifloxystrobin (25%) + tebuconazole (50%), Azoxystrobin (18.2%) + difenoconazole (11.0%), Carbendazim (12%) + mancozeb (63%) showed complete mycelial growth inhibition of *Aspergillus niger* causing black mould rot of garlic (Chavda and Brahmbhatt, 2016) [3].

Material and Methods

Fungal pathogens, were isolated from bits of infected skin of fruits. Bits were surface sterilized by dipping in mercury chloride solution (0.1%) for 2 minute followed by three washings with sterilized distilled water and transferred to sterilized Petri dishes containing potato dextrose agar (PDA) medium. The inoculated Petri dishes were incubated at 25 ± 1^0C in BOD incubator.

Tests were conducted to confirm the pathogenic ability of the isolates. Nagpur mandarin fruits were procured from market and inoculated with individual fungal pathogens *viz*., *Colletotrichum gloeosporioides*, *Aspergillus niger*, *P. digitatum*, *Geotrichum candidum* and *Trichoderma viride*. For each fungal isolate, three fruits were inoculated. The appearance of symptoms of rotting in inoculated fruits was observed periodically up to ten days of inoculation.

Double strength fungicides, oils and wax suspension were prepared in sterile distilled water. A highly virulent pathogens were were grown on PDA and at 10^{th} days old growth of the colony was scraped and suspended in sterile distilled water and spore suspension was prepared. A drop of spore and fungicidal suspension was placed on coverslip and inverted on cavity slide. The edges of coverslip were sealed with vaseline to avoid evaporation. In control, only water suspension was used and all the treatments were replicated thrice. The spores were scored as germinated if the germ tube length was equal or exceed that of the spore length (Suprapta *et al*., 1997) [17]. The slides were incubated at room temperature at 27 $\pm2^0$ C. Total number of spores and germinated spores were counted at (10 x) microscopic fields at an interval of 24, 48 and 72 h. The inhibition of spore germination was calculated in each treatment.

Results and Discussion

Effect of treatments on conidial inhibition of *Colletotrichum gloeosporioides* in Nagpur mandarin

Nine treatments were tested to see the efficacy of these treatments to restrict the conidial germination. After 24 hours, Carbendazim @ 0.1 percent was found superior in inhibiting the conidial germination (87.26%) of *C. gloeosporioides* at par with Benomyl @ 0.05 percent + Potassium sorbate @ 2% (83.44%), Benomyl @ 0.05 percent (83.01%), Hydrogen peroxide @ 10 percent (79.08%) whereas, lowest conidial inhibition was recorded in Neem oil (52.29%). After 48 hours, conidial inhibition (81.11%) was achieved in Carbendazim @ 0.1 percent at par with Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (77.61%), Benomyl @ 0.05 percent (77.27%) while Hydrogen peroxide @ 10 percent recorded inhibition (73.91%). Least conidial inhibition was recorded in Neem oil (44.44%). After 72 hours, *C. gloeosporioides* conidial inhibition was (75.90%) in Carbendazim @ 0.1 percent of which was at par with Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (74.07%), Benomyl @ 0.05 percent (73.68%), Hydrogen peroxide @ 10 percent inhibit (70.41%) and Neem oil @ 1% was found least effective against *C. gloeosporioides* with low conidial inhibition (40.63%). (Table 1).

Maximum conidial inhibition (91.04%) was recorded in Hydrogen peroxide @ 10 percent followed by Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (77.51%), Benomyl @ 0.05 percent (75.13%), *Saccharomyces cerevisiae* @ 10^8 cfu/ml inhibited (71.97%) while minimum inhibition was recorded in Neem oil (46.78%) at 24 hrs. After 48 hours, highest inhibition was recorded in Hydrogen peroxide @ 10 percent (84.76%) followed by Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (70.77%), Benomyl @ 0.05 percent (69.46%), however lowest inhibition (38.52%) was observed in Neem oil @ 1 percent. After 72 hours, Hydrogen peroxide @ 10 percent recorded maximum inhibition (77.59%), followed by Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (63.44%) whereas, Neem oil @ 1% was found to be less effective against *Geotrichum candidum* with inhibition of 40.63%. (Table 1).

The treatments were found effective to suppressing growth of *Aspergillus niger*. Conidial germination restricted up to 88.68% by Carbendazim @ 0.1 percent and was at par with Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (87.27%), Benomyl @ 0.05 percent inhibited (86.79%), however, Neem oil 1% was found least effective against *Aspergillus niger*, i.e. (39.84%). After 48 hours, maximum inhibition i.e. 84.78% was recorded in Carbendazim @ 0.1 percent, which was at par with Benomyl @ 0.05 percent with combination Potassium sorbate @ 2% (82.61%), Benomyl @ 0.05 percent (81.52%), Hydrogen peroxide @ 10 percent (80.04%), and lowest was observed in Neem oil @ 1% (48.91%). After 72 hours, highest inhibition of *Aspergillus niger* was (80.26%) observed in Carbendazim @ 0.1 percent, which was at par with each other Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% inhibited (78.67%), while lowest inhibition was observed in Neem oil @ 1 percent (33.56%). (Table 2).

Carbendazim @ 0.1 percent was found superior when observed at 24 hrs in inhibiting the conidial germination (92.88%) of *Penicillium digitatum* which was at par with Benomyl @ 0.05 percent with combination Potassium sorbate @ 2% (90.84%), Benomyl @ 0.05 percent (90.72%), whereas, lowest inhibition (40.72%) was observed in Neem oil. After 48 hours, maximum inhibition (86.44%) was achieved in Carbendazim 0.1 percent followed by Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (83.81%), however least inhibition was recorded in Neem oil i.e. 33.11%. After 72 hours, highest inhibition (80.83%) was recorded in Carbendazim @ 0.1 percent of *Colletotrichum gloeosporioides* which was at par with Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (79.26%), Benomyl @ 0.05 percent (79.14%), whereas, Neem oil 1% was found least effective against *Penicillium digitatum* exhibited low inhibition (28.16%) (Table 2).

Highest conidial inhibition (85.14%) of *Trichoderma viride* was achieved in Carbendazim 0.1 percent which was at par with Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% (83.27%), Benomyl @ 0.05 percent inhibited (82.79%), After 48 hours, maximum inhibition (78.32%) was recorded in Carbendazim @ 0.1 percent, which was at par with Benomyl @ 0.05 percent with combination of Potassium sorbate @ 2% inhibited (77.06%). Highest conidial inhibition of *Trichoderma viride* (72.53%) was observed in Carbendazim @ 0.1 percent, while lowest was observed in Neem oil @ 1 percent (30.32%) (Table 3). Similar results were reported earlier by Prabhakar *et al*. (2008) [12] who tested eight fungicides, Carbendazim (0.1%) inhibited the mycelial

growth and conidial germination of *C. gloeosporioides* of mango very effectively, followed by thiophanate-methyl (0.1%). El-Mougy *et al*. (2008) [6] found 1.5 to 2.00% concentrations of Hydrogen peroxide were able to reduce the complete linear growth and spore germination of *Botrytis cinerea, Rhizopus stolonifer, Penicillium digitatum* and *P. italicum.* The results are in agreement with Ladaniya and Singh (2000) [8] who stated that Nagpur mandarin fruits treated with Hydrogen peroxide (10%) for two min dip gave least decay incidence with a percentage score of 10.48% for 30 days of storage period at ambient conditions. It might be due to hydrogen peroxide produces reactive hydroxyl free radicals and ions that can attack membrane lipids, DNA and other essential cell compounds (Ralph, 2003) [13]. Present results also confirms the finding of Reddy (2004) [14] Chavda and Brahmbhatt (2016) [3] reported that Isoprothiolane, Trifloxystrobin (25%) + tebuconazole (50%), Azoxystrobin (18.2%) + difenoconazole (11.0%), Carbendazim and Carbendazim (12%) + mancozeb (63%) showed complete mycelial growth inhibition of *Aspergillus niger* causing black mould rot of garlic. Baria *et al*., (2016) [2] noticed that Carbendazim @ 0.1% inhibited the complete mycelial growth of *Fusarium pallidoroseum* which cause fruit rot in citrus. Wahab and Rashid (2012) [19] treated orange fruits with wax proved higher efficiency to control post-harvest rots than untreated fruits. Hagenmaier and Shaw (2002) [5] observed that waxing of citrus fruits after harvest enhance their shine and to reduce their weight loss and shrinkage. Gurjar (2011) [4] found that Carbendazim @ 0.2% and Carbendazim + mancozeb @ 0.2% produced maximum inhibition of mycelial growth of *Penicillium italicum* of kinnow. These results agreed with Wani and Taskeen-Un-Nisa (2011) [20] who found Carbendazim inhibited cent percent growth of *Aspergillus niger*.

Table 1: Effect of fungicides on conidial inhibition of post-harvest pathogens of Nagpur mandarin

Tr. No.	Treatment details	Conc. (%)	*C. gloeosporioides* (hrs)						*G. candidum* (hrs)					
			24		48		72		24		48		72	
			Av %	PI	Av %	PI	Av %	PI	Av %	PI	Av %	PI	Av %	PI
T1	Carbendazim	0.1	12.74 (4.88)*	87.26 (99.69)	17.04 (8.60)	81.11 (97.59)	24.10 (16.69)	75.90 (94.05)	38.46 (38.69)*	61.54 (77.28)	48.86 (56.71)	51.14 (60.62)	57.67 (71.36)	42.33 (45.35)
T2	Benomyl	0.05	16.99 (8.61)	83.01 (98.50)	22.73 (14.99)	77.27 (95.07)	26.32 (19.67)	73.68 (92.09)	24.87 (17.70)	75.13 (93.34)	30.54 (25.83)	69.46 (87.67)	39.88 (41.12)	60.12 (75.09)
T3	Benomyl + P. sorbet	0.05+2	16.65 (8.23)	83.44 (98.62)	22.39 (14.52)	77.61 (95.38)	25.93 (19.13)	74.07 (92.45)	22.49 (14.65)	77.51 (95.16)	29.23 (23.86)	70.77 (89.14)	36.56 (35.49)	63.44 (79.99)
T4	Hydrogen peroxide	10	20.92 (12.81)	79.08 (96.39)	26.09 (19.39)	73.91 (92.30)	29.59 (24.39)	70.41 (88.69)	8.96 (2.44)	91.04 (99.89)	15.24 (6.93)	84.76 (99.09)	22.41 (14.55)	77.59 (95.36)
T5	Neem oil	1	47.71 (54.72)	52.29 (62.56)	55.56 (68.01)	44.44 (49.03)	59.38 (74.05)	40.63 (42.40)	53.22 (64.14)	46.78 (53.10)	61.48 (77.19)	38.52 (38.79)	67.17 (84.93)	32.83 (29.40)
T6	Eucalyptus oil	1	45.10 (50.17)	54.90 (66.93)	51.11 (60.58)	48.89 (56.76)	56.84 (70.07)	43.16 (46.79)	49.93 (58.56)	50.07 (58.80)	57.33 (70.85)	42.67 (45.94)	63.56 (80.16)	36.44 (35.29)
T7	Wax	6	35.95 (34.47)	64.05 (80.74)	44.44 (49.02)	55.56 (67.95)	51.58 (61.38)	48.42 (55.95)	32.29 (28.55)	67.71 (85.56)	36.88 (36.02)	63.12 (79.55)	42.81 (46.18)	57.19 (70.63)
T8	*Saccharomyces cerevisiae*	10^9	33.99 (31.26)	66.01 (83.46)	41.30 (43.56)	58.70 (73.00)	51.58 (61.38)	48.42 (55.93)	28.031 (22.10)	71.969 (90.35)	35.28 (33.37)	64.72 (81.71)	41.84 (44.50)	58.16 (72.16)
T9	Control		100 (96.51)	-	100 (96.30)	-	100 (96.51)	-	100 (96.05)	-	100.00 (96.51)	-	100.00 (96.05)	42.33 (45.35)
	SE (m) ±		1.07	1.12	1.36	0.99	1.01	0.96	1.13	1.14	1.16	1.05	1.28	1.29
	CD (P = 0.01)		4.12	4.31	5.22	3.81	3.87	3.67	4.34	4.39	4.46	4.02	4.93	4.97

*Figures in parenthesis are arc sin values
Average of three replications

Table 2: Effect of fungicides on conidial inhibition of post-harvest pathogens of Nagpur mandarin

Tr. No.	Treatment details	Conc. (%)	*Aspergillus niger* (hrs)						*Penicillium digitatum* (hrs)					
			24		48		72		24		48		72	
			Av %	PI	Av %	PI	Av %	PI	Av %	PI	Av %	PI	Av %	PI
T1	Carbendazim	0.1	11.32 (3.87)*	88.68 (99.93)	11.32 (6.91)	84.78 (99.15)	11.32 (11.42)	80.26 (97.12)	7.12 (1.56)*	92.88 (99.73)	13.56 (5.52)	86.44 (99.59)	19.37 (11.02)	80.63 (97.22)
T2	Benomyl	0.05	13.21 (5.24)	86.79 (99.61)	13.21 (10.11)	81.52 (97.81)	13.21 (14.87)	77.33 (95.17)	9.28 (2.62)	90.72 (99.96)	16.27 (7.87)	83.73 (98.79)	20.86 (12.69)	79.14 (96.43)
T3	Benomyl + P. sorbet	0.05+2	12.73 (4.87)	87.27 (99.75)	12.73 (8.95)	82.61 (98.35)	12.73 (13.25)	78.67 (96.12)	9.16 (2.55)	90.84 (99.96)	16.19 (7.79)	83.81 (98.82)	20.74 (12.56)	79.26 (96.51)
T4	Hydrogen peroxide	10	15.09 (6.80)	84.91 (99.19)	15.09 (11.24)	80.43 (97.22)	15.09 (13.87)	78.15 (95.76)	12.09 (4.41)	87.91 (99.85)	19.48 (11.14)	80.52 (97.27)	26.57 (20.02)	73.43 (91.85)
T5	Neem oil	1	39.84 (41.05)	60.16 (75.23)	39.84 (60.54)	48.91 (56.80)	39.84 (84.01)	33.56 (30.57)	59.28 (73.89)	40.72 (42.56)	66.89 (84.58)	33.11 (29.85)	71.84 (90.27)	28.16 (22.28)
T6	Eucalyptus oil	1	37.74 (37.47)	62.26 (78.33)	37.74 (57.75)	50.54 (59.60)	37.74 (79.50)	36.91 (36.07)	56.81 (70.03)	43.19 (45.11)	63.59 (80.20)	36.41 (35.24)	58.13 (72.11)	41.87 (44.55)
T7	Wax	6	30.19 (25.30)	69.81 (88.07)	30.19 (39.83)	60.87 (76.29)	30.19 (54.06)	52.67 (63.22)	24.39 (17.07)	75.61 (93.81)	32.36 (28.66)	67.64 (85.51)	39.23 (40.00)	60.77 (76.14)
T8	*Saccharomyces cerevisiae*	10^9	33.33 (30.20)	66.67 (84.30)	33.33 (32.97)	64.96 (82.07)	33.33 (49.42)	55.33 (67.64)	26.87 (20.44)	73.13 (91.56)	34.53 (32.14)	65.47 (82.75)	41.34 (43.63)	58.66 (72.94)
T9	Control		100 (96.30)	-	100 (96.68)	0.00	100 (96.68)	-	100 (96.81)	-	100.00 (96.68)	-	100.00 (96.05)	-
	SE (m) ±		0.98	0.53	0.95	0.63	0.88	0.63	0.70	0.53	0.82	0.58	1.11	0.67
	CD (P = 0.01)		3.77	2.02	3.66	2.43	3.39	2.64	2.68	2.05	3.13	2.24	4.25	2.56

*Figures in parenthesis are arc sin values
Average of three replications

Table 3: Effect of fungicides on conidial inhibition of post-harvest pathogen of Nagpur mandarin

Tr. No.	Treatment details	Conc. (%)	*Trichoderma viride* (hrs)					
			24		48		72	
			Av %	PI	Av %	PI	Av %	PI
T1	Carbendazim	0.1	16.33 (7.92)*	83.67 (98.77)	23.91 (15.15)	76.09 (94.20)	29.22 (23.84)	70.78 (89.15)
T2	Benomyl	0.05	18.37 (9.95)	81.83 (39.30)	25.71 (17.47)	74.29 (92.65)	33.12 (29.89)	66.88 (84.57)
T3	Benomyl+ Potassium sorbet	0.05+2	17.65 (9.21)	82.35 (98.21)	23.85 (16.36)	76.15 (94.25)	31.82 (27.81)	68.18 (86.17)
T4	Hydrogen peroxide	10	20.41 (12.18)	79.59 (96.72)	27.27 (22.43)	72.73 (91.12)	35.06 (33.00)	64.94 (82.04)
T5	Neem oil	1	57.14 (70.53)	42.86 (46.27)	67.39 (83.96)	32.61 (29.05)	74.68 (93.00)	25.32 (18.31)
T6	Eucalyptus oil	1	53.06 (63.88)	46.94 (53.38)	64.13 (82.31)	35.87 (34.34)	71.43 (89.84)	28.57 (22.88)
T7	Wax	6	51.02 (60.43)	48.98 (56.92)	34.78 (34.18)	65.22 (82.42)	44.16 (48.53)	55.84 (68.47)
T8	*Saccharomyces cerevisiae*	10^9	42.86 (46.27)	57.14 (72.13)	53.26 (62.54)	46.74 (53.03)	63.64 (80.27)	36.36 (33.50)
T9	Control		100 (96.30)	-	100.00 (96.98)	-	100.00 (96.30)	-
	SE (m) ±		1.14	0.70	0.96	0.77	1.18	0.75
	CD (P = 0.01)		4.38	2.70	3.70	2.97	4.52	2.89

*Figures in parenthesis are arc sin values
Average of three replications

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